THE RHIZOCTONIA BROWN ROT AND OTHER FRUIT ROTS OF STRAWBERRIES

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THE RHIZOCTONIA BROWN ROT AND OTHER FRUIT ROTS OF STRAWBERRIES[1]

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INTRODUCTION

The fruit rots of strawberries have received much less attention than the importance of the crop and the great losses from decay would seem to justify. A number of fungi are known which attack strawberries and it is to be expected that as investigation continues there will be discovered numerous other species which in certain localities or under some conditions are capable of causing serious fruit rots. Very recently Rose [2] has published the results of an extensive study of a fruit rot of strawberries found to be destructive in the Arkansas and Tennessee regions and which he calls "leather rot." In the present paper the writers report a new or little known field rot which is very important in Florida. Since both these diseases produce a brown discoloration, and at least two other so-called "brown rots" of strawberries are already known, it seems necessary to briefly discuss at this time the distinguishing characters of these rots.

THE BROWN RHIZOCTONIA ROT

Each season since 1920 as strawberries have come into the Washington market from Florida the writers have observed a peculiar rot on many of the berries which appeared to be perfectly sound on one side. Some of these berries were deformed as though one side had failed to fill out properly. This part of the berry was commonly affected with a somewhat dry brown rot which often had a blackish color, due chiefly to the adhering black sandy soil. This suggested that the rot might be due to some soil organism.

Study of the rots in Florida strawberry fields, especially in the vicinity of Plant City in 1923, and Kissimmee in 1924, shows that while Botrytis appears in epidemics, suddenly and with very destructive effects during or soon after brief periods of rainy weather, the rot just mentioned as found in the Washington markets develops much more regularly in the fields and makes it necessary to cull out large numbers of berries during picking and packing. As this disease is almost always present, yet rarely if ever epidemic, it attracts relatively little attention among growers. Continued observation has convinced the writers, however, that it is a constant source of loss to strawberry growers in central Florida and, until a remedy is discovered, will remain a handicap to the industry. Careful observation has convinced the writers that Rhizoctonia caused at least half the loss from field rots in this region during the season of 1923–24.

The characters of this rot are so pronounced that it can be distinguished readily from the other so-called brown rots of strawberry. Affected berries are generally one-sided, and show a hard brown rot, to which often adhere quantities of sandy soil. Decay regularly starts on the under side of the berry where it comes in contact with the soil, and a small amount of soil will usually be found clinging to the decayed spot even in dry weather. If the soil is of the black

[1] Received for publication Feb. 25, 1924

[2] Rose, D. H. LEATHER ROT OF STRAWBERRIES. Jour. Agr. Research, 28: 357-376, illus. 1924.

sandy type common in low places in many Florida strawberry fields, the surface of the rotting area will of course appear blackish (Pl. 1, A, C). If little or no soil clings to the berry, the true dark brown color of the rot will be evident (Pl. 1, D). The rot usually starts before the berry begins to turn red, sometimes even before the fruit is a third grown (Pl. 1, E). In this case the discolored side will be of a lighter brown, and only a little soil will adhere. An early attack on the berry will result in a deformed fruit, but as the rot progresses rather slowly, the upper side of the berry develops normally and ripens without showing rot, so that one can not tell that the berry is diseased until it is turned over. As a result many such berries are picked unintentionally and only extreme care will prevent their being overlooked by packers.

Sections of the rotting berry show that there is a definite line of demarcation in the pulp indicating clearly how far the disease has spread. Where healthy and diseased tissues meet, the pulp is only slightly faded and brownish; otherwise the diseased area stands out sharply on account of its rich brown color. The rotted part later becomes dried out or mummified (Pl. 1, B). By using a hand lens one is able to see masses of hyphae which bind the particles of soil together and hold the dirt to the surface of the berry. It will be shown that these soil hyphae belong to the Rhizoctonia which is causing the rot. In a free-hand section of the browning pulp examined under the microscope can be seen vast numbers of hyphae crowding in between the cells in the more recently invaded regions (Pl. 2, *B*, *a*), while in those parts of the berry where the fungus has completed its work the cells are crushed and distorted and the spaces are packed with felty masses of mycelium. If the affected part of the berry is cut away the remainder will be found to be perfectly edible (Pl. 1, C), Such rot fungi as Botrytis and Rhizopus grow through the berry and usually completely destroy it once they have gained a foothold. The appearance of berries attacked by the hard rot is such that they will be discarded wherever found. The rot develops so slowly that it would not be communicated rapidly to other berries under refrigeration in transit.

Whether the fungus, as it advances between the cells, attacks only the middle lamellae, as is so frequently reported for other intercellular fungi, is uncertain. At first the cells of the invaded tissues actually become hypertrophied, consequently the intercellular hyphae are put under pressure and are compelled to force back the elastic cell walls as they advance (Pl. 2, B, *c*). Certainly water is not withdrawn from the pulp cells in mass, resulting either in a wet rot such as is produced by Rhizopus or in a separation or falling apart of host cells, such as follows the work of Pezizella. Water seems to be lost more by a drying out process. Cells in the decaying tissues gradually collapse after the period of swelling and the hyphae crowd in, becoming more and more intracellular. The feeding of the hyphae in the middle lamellae may be the cause of a sort of mucilaginous disorganization which is manifested by the presence of quantities of intercellular stainable substance which so often surrounds the hyphae. This is more noticeable as the disease progresses. Striae or laminations in the adjacent walls are then more distinct. The intercellular substance and the peculiar manner in which the mucilaginous layers are split apart are brought out in Plate 2, figure B, *b*. The contents of the unstained hyphae are foamy or vacuolate.

MYCELIAL CHARACTERS

The mycelium of the fungus is comprised of septate branching hyphae of fairly uniform diameter, though where a branch grows out the diameter is somewhat less. In some cases hyphae are very coarse for short distances and branches are very irregular with blunt ends. The cells usually contain more than one

nucleus, sometimes as many as five; two or three being the common numbers. The nuclear wall is not very distinct in stained preparations but the large nucleole is always plainly visible, surrounded by a clear place in the cytoplasm (Pl. 2, A, *b*).

ARTIFICIAL INOCULATIONS ON STRAWBERRIES

Strawberries growing in pots in the greenhouse at the Arlington experiment farm, Virginia, were readily inoculated by laying pieces of agar bearing mycelium on the soil beneath half-grown berries. In every case the berries developed the characteristic rot within three or four days. The fungus penetrated the soil at the point attacked so that when the berry was removed some of the soil was attached to it. The fungus was recovered from such diseased berries by transfer of pulp from points well below the surface. Greenish berries from the Washington market have also been inoculated in damp chambers by placing bits of agar covered with hyphae from test-tube cultures on the bottom of damp chambers and then laying an apparently sound berry on the piece of agar. The brown rot developed in every case within two days (Pl. 1, G). Since the epidermis of a strawberry is so liable to rupture from all sorts of causes it is not certain that the fungus actually penetrated the unbroken epidermis.

Sections of berries artificially inoculated show that the hyphae crowd in between the akene and that part of the receptacle in which the akene is partly sunken. It may be that the fungus finds a film of moisture in this region where the walls of the epidermis are also easily penetrated. The most noticeable effect upon the host tissue is a swelling and clearing of the cells between which the hyphae are pushing. The cell contents take very little stain. The fungus apparently does not seek out any particular part of the berry. Hyphae are found in every tissue in the region attacked. The sieve tubes or long conducting cells of the vascular bundles leading to the "seeds" are pushed aside and hyphae crowd in between them or run along between these cells in all directions (Pl. 3, A). Hyphae also pack in between the small cells of the pulp between the akenes and crowd up close to the cells of the sclerotized and thickened cell walls (Pl. 3, B). No living tissue appears to be immune. If nuclei were present in the diseased pulp cells examined, they did not take the stain. The nuclei of the fungus, however, appeared very distinctly (Pl. 2, A, *b;* B).

THE TAN BROWN ROT OF STRAWBERRIES

An excellent description of the Pezizella brown rot of strawberries was published by Stevens and Peterson[3] in connection with their account of the organism causing the disease. They described this fungus as *Patellina fragariae* although it had already been described under a great many different names.[4] The region attacked by the fungus is marked on green berries by the appearance of small sunken spots. These spots are tan colored, slightly sunken, and enlarge only slowly. When a ripe berry is attacked, the rot spreads much more rapidly but the color of the spot is about the same (Pl. 1, H). The fungus grows into the pulp of the berry so that the rotted portion is deeper than its diameter. The core of the region attacked is consumed and the host tissue is replaced by mycelium so that the decayed portion finally presents a spongy dry texture. The host tissue immediately surrounding the diseased area, although devoid of mycelium, becomes soft and wet and its cells separate from each other. As the core of the rotten spot is very tenacious it can easliy be removed intact. This

[3] STEVENS, F. L., and PETERSON, A. SOME NEW STRAWBERRY FUNGI. Phytopathology. 6: 258-267, illus. 1916.

[4] SHEAR, C. L., and DODGE, B. O. THE LIFE HISTORY AND IDENTITY OF "PATELLINA FRAGARIAE," "LEPTOTHYRIUM MACROTHECIUM" AND "PEZIZA OENOTHERAE," Mycologia, 13: 135-170. pl. 8-10. 1921.

characteristic serves for the ready identification of this rot. In addition it is often possible to see with the aid of a hand lens the little "sporodochia" of the Hainesia (Patellina) stage of the fungus on the surface of the spot. Teased and microtome sections show, according to Stevens and Peterson, that the host cells are filled with hyphae.

THE LEATHER ROT OF STRAWBERRIES

The leather rot, as Rose[5] calls the disease, is caused by a Phytophthora very similar to *P. cactorum* and is characterized by a definite though slight softening of the affected tissues and by internal discoloration. It is a typical field rot attacking berries in all stages of growth, and is most common following rainy periods, a characteristic which results in the name "water soak" often used by growers. The results of Rose's inoculation experiments did not lead him to conclude that the hyphae of this Phytophthora can penetrate the unbroken strawberry epidermis. In the field, however, berries touching the ground were first affected on the under side, suggesting that the fungus may be a true soil organism, and that it may be able to penetrate the unbroken epidermis under favorable circumstances.

Green fruit becomes brown where attacked, and fruit that has turned red before the fungus gains entrance presents a series of color changes as the disease progresses. At the center of the spot the color becomes yellow to light brown, spreading out from here the color changes to purple and then to the natural red as sound tissue is approached (Pl. 1, J). The most characteristic feature of the rot is shown in sections of the fruit. The vascular tissue becomes markedly discolored, being a much deeper brown than the affected pulp (Pl. 1, K). In the early stages of the disease vascular browning may be the only symptom. There is no clear line of demarcation between sound and diseased tissue, such as is characteristic of the hard brown rot. Neither can the diseased tissue be scooped out as it can be in the case of the Pezizella rot. One striking characteristic of leather rot is that even slightly rotten berries are bitter to the taste.

THE BOTRYTIS BROWN ROT OF STRAWBERRIES

Strawberries attacked by Botrytis present symptoms which in the early stages of the disease are quite different from those shown in the later stages. As the hyphae penetrate into the pulp it loses its natural color and turns light brown. Later the color becomes somewhat darker (Pl. 1, I). The rotting pulp is at first rather soft, almost watery, though never leaky. This stage is soon superseded by a drying out so that the berry becomes firm. After the disease has run its course, the berry is found to be hard and dry,[6] which results in the use of the term "dry rot" among growers. The tendency, however, to make use of color characters describing rots will always prevail to a certain extent, so that shippers and inspectors no doubt will continue to use the term brown rot for this and similar diseases.

As soon as aerial hyphae with their crop of conidia develop, the surface of the berry presents a characteristic appearance which is referred to as "gray mold." Sections of immature or green berries recently affected with Botrytis rot do not show a distinct line of demarcation between healthy and diseased tissue. The diseased pulp is of a darker brown near the surface. The color shades off to a lighter brown and ends with the natural color of the healthy fruit in parts not invaded by the mycelium. Stevens [7] who investigated the host parasite relation

[5] ROSE, D. H. LEATHER ROT OF STRAWBERRIES. Jour. Agr. Research, 28: 1924.

[6] STEVENS, NEIL E., and WILCOX, R. B. FURTHER STUDIES OF THE ROTS OF STRAWBERRY FRUITS. U. S. Dept. Agr. Bul. 686, 14 p. 1918. Literature cited, p. 14.

[7] STEVENS, NEIL E. PATHOLOGICAL HISTOLOGY OF STRAWBERRIES AFFECTED BY SPECIES OF BOTRYTIS AND RHIZOPUS. Jour. Agr. Research 6: 361-366, pl. 49-50. 1916.

in connection with this disease found that the hyphae penetrate the cell walls readily and dissolve the middle lamellae. The hyphae were found to grow between the cells for some distance, then to penetrate the cell walls becoming intracellular. All parts of the berry are subject to attack by the fungus, which if allowed to develop does not stop at localized infections. It is evident from the way the cytoplasm shrinks away from the cell walls and becomes disorganized that the host cells are killed quickly. Water is so slowly and gradually withdrawn from the host cells that it evaporates from the surface of the berry and no leak is caused, in spite of the fact that hyphae penetrate pulp or storage cells everywhere. The rotting berry thus becomes dried and mummified.

COMPARISON OF THE FOUR BROWN ROTS

The two field rots most likely to be confused with the hard brown rot are those caused by Botrytis and Pezizella, although each has sufficiently distinctive characters to make indentification reasonably certain. The Botrytis rot is a lighter brown and more watery at the beginning. That caused by Pezizella forms a pocket in the sticky scablike rotted portion. In only one case out of 126 cultures made by direct transfer of pulp from berries selected in the field as affected with hard brown rot was Botrytis obtained as a result of faulty diagnosis. The hard rot caused by the Rhizoctonia under discussion in this paper is easily distinguished from the tough dry rot due to Botrytis by the clear line of demarcation between healthy and diseased tissue characteristic of the Rhizoctonia rot.

The Rhizoctonia hard brown rot and the Pezizella soft brown rot are alike in that in both there is a distinct line separating the diseased from the healthy tissue. Not infrequently berries infected with the Rhizoctonia when shipped arrive at destination with the uninfected portion of the berry still edible. A berry infected with Botrytis or Phytophthora when shipped is usally so rotten as to be wholly inedible on arrival. The leather rot (Phytophthora rot) is the only one of the four brown rots to cause a marked vascular discoloration.

The surface of berries affected with Phytophthora under moist conditions becomes covered with a pure white growth of hyphae and conidiophores (Pl. 1, J). Those affected with Botrytis develop the gray mold which consists in part of branched conidiophores. The Pezizella rot develops little disc-shaped fruiting bodies sometimes called sporodochia. The Rhizoctonia hard rot develops only light brown, fluffy hyphae.

The mycelium of Botrytis penetrates the host cells in every direction and is both inter- and intracellular. The hyphae of the hard brown rot fungus are typically intercellular, but they are often found within the cells. Hyphae of the Pezizella are probably capable of penetrating the walls of the pulp cells but are not known to penetrate the cutinized epidermis. Little is known as to the manner in which the hyphae of the leather rot attack the host cell.

KNOWN DISTRIBUTION OF THE VARIOUS BROWN ROTS

The Rhizoctonia rot is now known from central Florida, North Carolina and possibly from Tennessee. Leather rot has been found in Mississippi, Louisiana, Arkansas, Tennessee, Missouri, Kentucky, and Illinois. Pezizella rot has been observed by the writers in Cuba, Louisiana, Florida, Arkansas, Virginia, Maryland, Wisconsin, and Alaska. Botrytis has been found to some extent as a fruit rot of strawberries in every strawberry region visited, though it varies greatly in abundance.[8] It is serious in Alaska[9] and during many seasons in New England, and may become abundant in more southern regions during wet weather. Botrytis is also known to cause rot of strawberries in England.

[8] STEVENS, NEIL E. ROTS OF EARLY STRAWBERRIES IN FLORIDA AND SOUTHERN CALIFORNIA. Amer. Jour. Bot., 9: 204-211. 1922. Literature cited, p. 211.

[9] ANDERSON, J. P., BOTRYTIS CINEREA IN ALASKA. Phytopathology. 14: 152-155. 1924.

CONTROL

Investigations as to the possibility of the control of the various field rots of strawberries by spraying and dusting are now in progress. The results which have apparently been obtained by a few growers indicate the possibility of success but it will be several years before definite recommendations can be made. Covering the ground with a mulching of pine needles is occasionally practiced in Florida, and apparently results in a lessening of the rot caused by the Rhizoctonia.

SUMMARY

A fruit rot of strawberries found to be important in central Florida is caused by a fungus which in its mycelial characters resembles *Rhizoctonia solani* Kühn as commonly found on cultivated plants.

When the berry is first attacked by this fungus the cells of the invaded tissues become somewhat hypertrophied. Later the cells of the decaying tissues gradually collapse and the mycelium of the fungus which is at first intercellular becomes both inter and intracellular.

The "hard brown rot" caused by this Rhizoctonia is characterized by the fact that it regularly starts on the under side of the berry, advances slowly and shows a definite line of demarcation between the brown decayed portion and the normal uninfected pulp. In addition the berry is often distorted or "one-sided" in shape and particles of soil usually adhere tightly to the affected area.

The "tan rot" caused by *Pezizella lythri* is confined to a somewhat cone-shaped region, the base of which appears as a tan-colored, slightly sunken spot on the side of the berry. This rotted portion may readily be separated almost intact from the sound tissue.

The leather rot, caused by a species of Phytophthora is easily distinguished, if the fruit is cut open, by the fact that the vascular tissue becomes markedly discolored being a much deeper brown than the affected pulp. Berries rotted by this fungus are very bitter to the taste.

The Botrytis or grey mold rot is not localized in any part of the berry as the fungus grows readily in all tissues. Berries affected by Botrytis are brown and rather tough without either the marked vascular discoloration or localized decay which are characteristic of one or more of the other brown rots discussed in this paper.

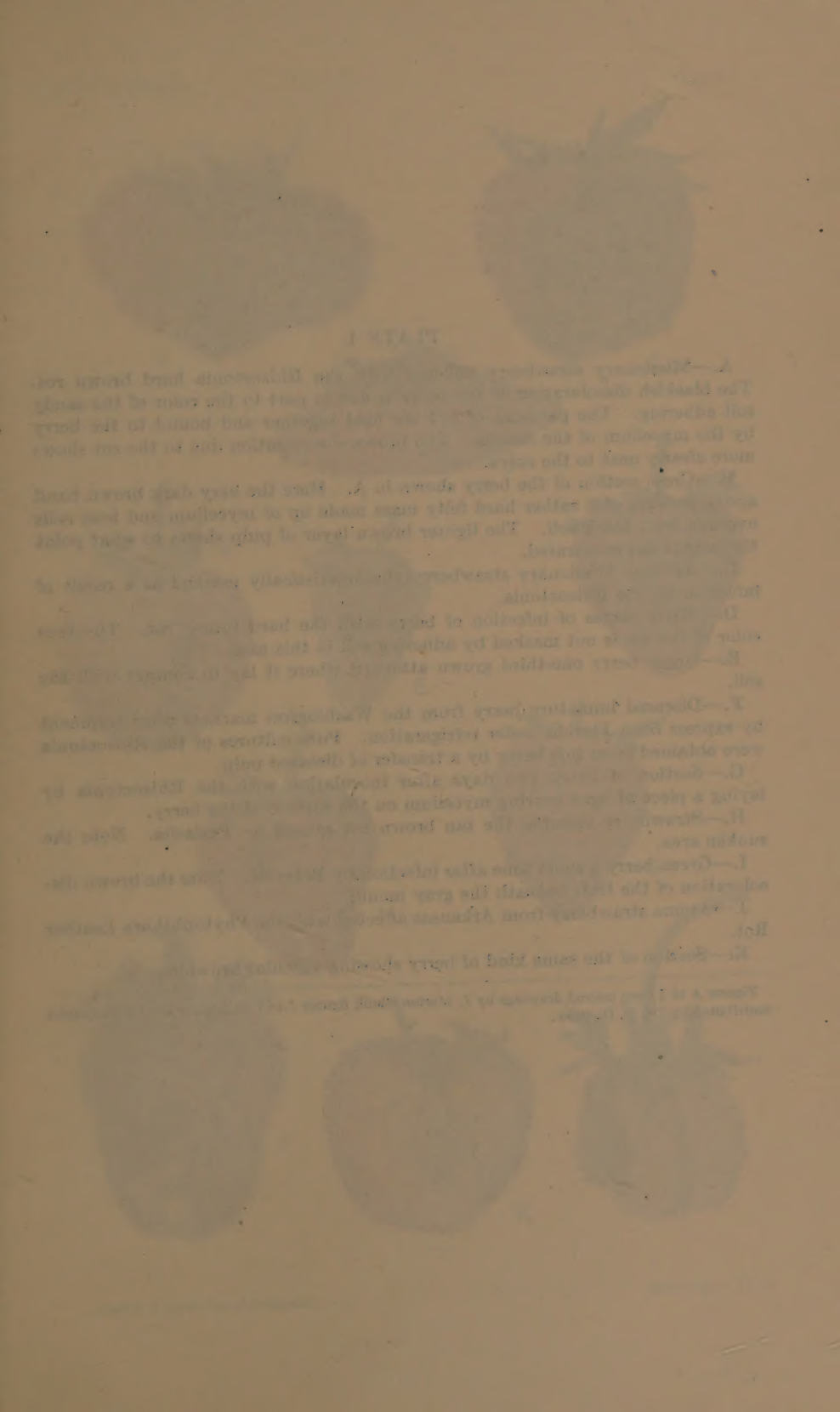


PLATE 1

A.—Missionary strawberry affected with the Rhizoctonia hard brown rot. The blackish discoloration of the berry is due in part to the color of the sandy soil adhering. The particles of soil are held together and bound to the berry by the mycelium of the fungus. The brown discoloration due to the rot shows more clearly next to the calyx.

B.—Cross section of the berry shown in A. Here the very dark brown band above consists of a rather hard felty mass made up of mycelium and host cells overlaid with black soil. The lighter brown layer of pulp shows to what point the fungus has penetrated.

C.—Another Missionary strawberry characteristically marked as a result of infection by the Rhizoctonia.

D.—Early stages of infection of berry with the hard brown rot. The true color of the rot is not masked by adhering soil in this case.

E.—Green berry one-third grown attacked where it lay in contact with the soil.

F.—Diseased immature berry from the Washington markets after shipment by express from Florida under refrigeration. Pure cultures of the Rhizoctonia were obtained from this berry by a transfer of diseased pulp.

G.—Section of berry two days after inoculation with the Rhizoctonia by laying a piece of agar bearing mycelium on the surface of the berry.

H.—Strawberry showing the tan brown rot caused by Pezizella. Note the sunken area.

I.—Green berry a short time after infection by Botrytis. Note the brown discoloration of the fruit beneath the gray mould.

J.—Aroma strawberry from Arkansas affected with the Phytophthora Leather Rot.

K.—Section of the same kind of berry showing vascular browning.

Figures A to I from colored drawings by J. Marion Shull; figures J and K, from colored photographs contributed by Dr. D. H. Rose.


A
B
C
D
E
F
G
H
I
J
K


B D
NATURAL


a
b
A

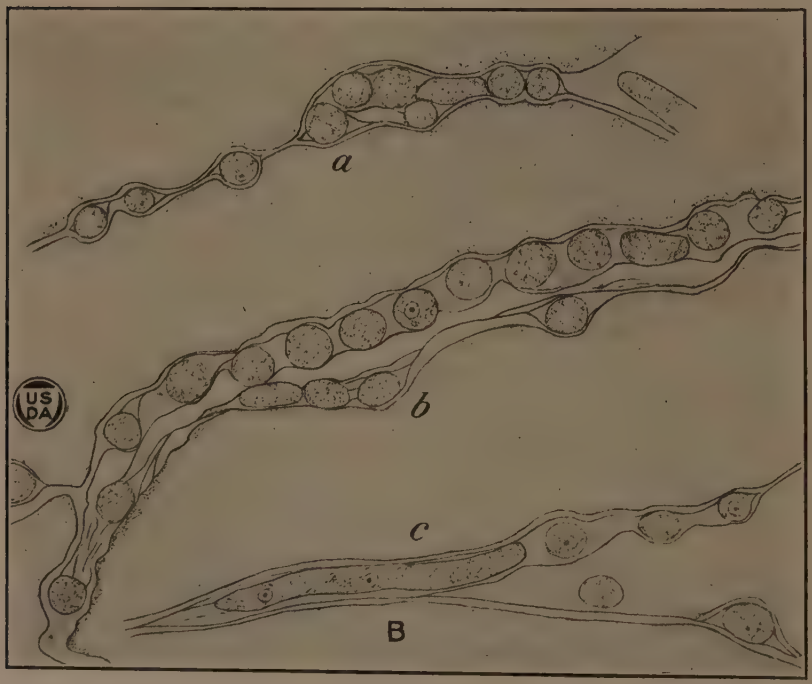

a
b
c
B

PLATE 2

Mycelium of the Rhizoctonia from a sclerotium and from the large pulp cells of Missionary strawberry, drawn by the aid of the camera lucida A. Zeiss 4 mm. 8 oc.; B, C. 1.5 mm. Obj., 8 oc. Reduced about one-third.

A.—(*a*) Terminal branches from a sclerotium, unstained. Most of the cells in this case contain one or two large oil globules and several smaller ones. (*b*) Intracellular hyphae showing character of branching, crescent-shaped hyaline areas at the cross walls and two or three nuclei in each cell. Stained with Flemming's triple stain.

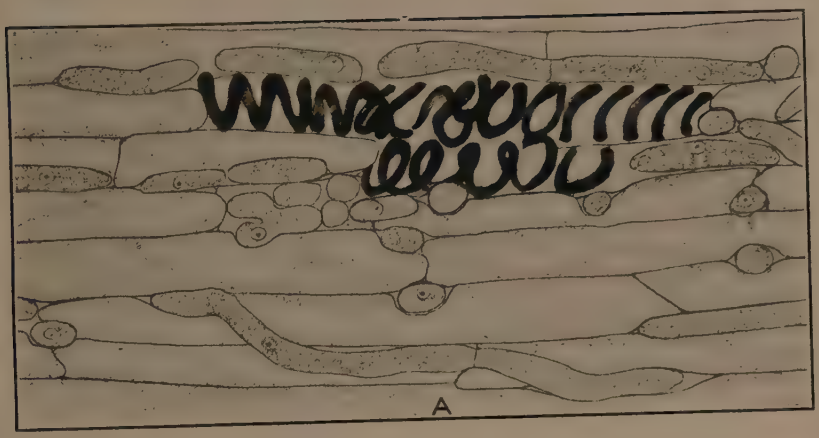
B.—(*a*) Intercellular hyphae in cross section pushing back the elastic walls of the turgid pulp cells. Note the intercellular substances, possibly derived from disorganized middle lamellae; (*b*) similar to the preceding except that the intercellular substances appear to be in more or less distinct layers. Cytoplasmic contents of pulp cells indicated by stippling. Large vacuoles filled with cell sap. Intercellular substance or disorganizing laminae of the walls shown by wash drawing; (*c*) hyphae pushing forward between two cells which at the right have spread apart to form an open intercellular space.

PLATE 3

Mycelium of the Rhizoctonia in the tissues of Missionary strawberry. Drawn with the aid of a camera lucida, Zeiss, 1.5 mm. Obj., 8 oc. Reduction about one-third.

A.—Section along the edge of a vascular bundle in the pulp. Hyphae push in between the long conducting cells forcing their walls aside. No intercellular spaces are present owing to the turgescence of the affected cells. Hyphae penetrate the vascular tissues in all directions.

B.—Section just beneath an akene, showing how the very small pulp cells in this region are entirely surrounded by intercellular hyphae which push up closely to the fruit or seed coats whose cells are thickened or sclerotized. No nuclei are visible in the host cells.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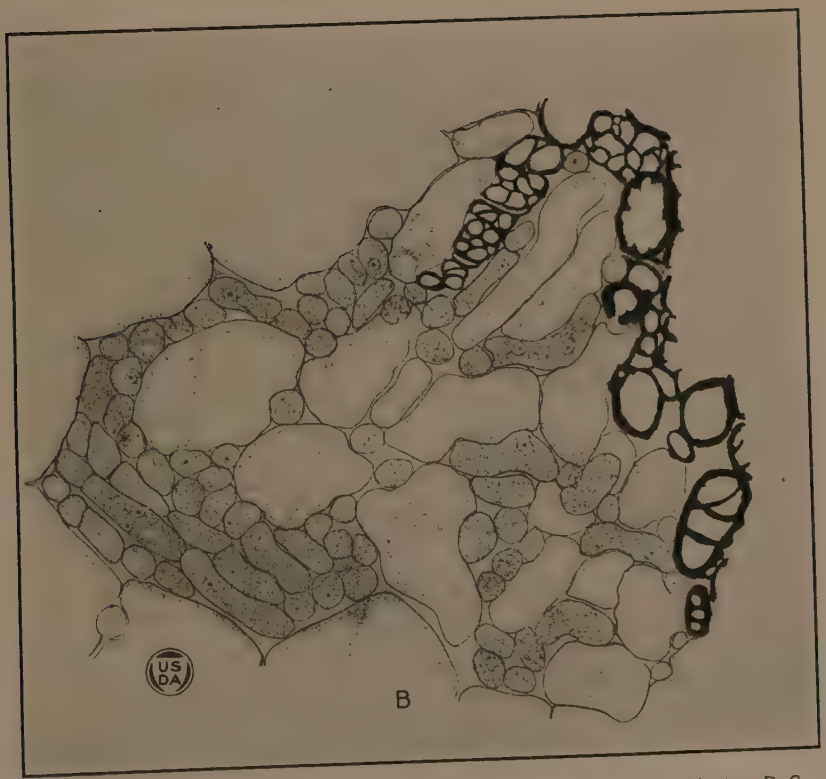
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B